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Attorney Docket No. 3523 P 002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re U.S. Patent Application of Wilhelm Schwaeble and Robert Braidwood Sim	)	
Application No.: 09/316,163	)	Examiner: Marianne DeBrino
Filed: June 18, 2001 (CPA filing date)	)	Group Art Unit: 1644
For: COMPLEMENT INHIBITOR	)	

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SUPPLEMENT TO
RESPONSE TO OFFICE ACTION MAILED MARCH 25, 2003

Further, in response to the Office Action mailed March 25, 2003, the applicant encloses a certified copy of the priority document.

The Commissioner is hereby authorized to charge any deficiency in fees to Deposit Account No. 23-0280.

Respectfully submitted,

Date: November 3, 2003

By: Monique A. Morneault

Monique A. Morneault, Reg. No. 37,893
Wallenstein Wagner & Rockey, Ltd.
311 South Wacker Drive, 53rd Floor
Chicago, Illinois 60606
Phone: (312) 554-3300
Attorneys for Applicants

MAM/183986.1

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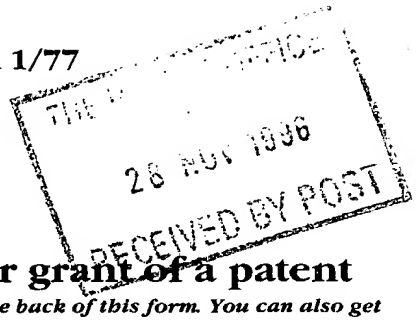

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Cardiff Road
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1. Your reference

M96/0591/GB

2. Patent application number
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9624731.7

28 NOV 1996

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University of Leicester
University Road
Leicester
LE1 7RH

Patents ADP number *(if you know it)*

7983480012

If the applicant is a corporate body, give the country/state of its incorporation

Great Britain

4. Title of the invention

COMPLEMENT INHIBITOR

5. Name of your agent *(if you have one)*

McNeight & Lawrence

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Regent House
Heaton Lane
Stockport
Cheshire
SK4 1BS

Patents ADP number *(if you know it)*

0001115001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

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a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

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Request for preliminary examination and search *(Patents Form 9/77)*	one
Request for substantive examination *(Patents Form 10/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature McNeight & Lawrence Date 27.11.96

12. Name and daytime telephone number of person to contact in the United Kingdom David L McNeight 0161 480 6394

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Statement of inventorship and of right to grant of a patent

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1. Your reference — M96/0591/GB

2. Patent application number *(if you know it)* — 9624731.7

3. Full name of the or of each applicant — University of Leicester

4. Title of the invention — Complement Inhibitor

5. State how the applicant(s) derived the right from the inventor(s) to be granted a patent — by virtue of employment

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7. I/We believe that the person(s) named over the page *(and on any extra copies of this form)* is/are the inventor(s) of the invention which the above patent application relates to.

Signature: McNeight & Lawrence — Date: 12.12.96

8. Name and daytime telephone number of person to contact in the United Kingdom — David L McNeight 0161 480 6394

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Enter the full names, addresses and postcodes of the inventors in the boxes and underline the surnames

Wilhelm Schwaeble
13 Swithland Court
Woodhouse Eves
Leicestershire LE12 8SJ

Patents ADP number *(if you know it)*: 7111206001

Robert Braidwood Sim
106 Water Eaton Lane
Gosford
Kidlington
Oxfordshire OX5 2PR

Patents ADP number *(if you know it)*: 7130944001

Patents ADP number *(if you know it)*:

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Complement Inhibitor

The present invention concerns regulation of complement activation, in particular the fluid phase regulation of complement activation.

The complement system (see McAleer, M.A. and Sim, R.B. in Activators and Inhibitors of Complement, Kluwer Academic Publishers, Dordrecht, ed R.B. Sim, 1993, p. 1-15; Reid, K.B.M. and Law, A., 1988, Complement, IRL Press, Oxford) is concerned with host defence against infection - upon activation of the system a catalytic set of reactions and interactions occur resulting in the targeting of the activating cell, organism or particle for destruction. Due to the destructive nature of the system it has the potential to cause severe damage to a host system if incorrectly triggered (Davis, A.E., 1988, Ann. Rev. Immunol., 6: 595-628; Frank, M.M., 1993, In: Complement in Health and Disease, 2nd Edition, Whaley, K. *et al.* eds., Kluwer Academic Publishers, Dordrecht, p. 229) and if its activity is diminished then it has the potential to leave the host open to attack from infecting pathogens.

This is particularly the case with patients suffering from Factor H (FH) deficiency which leads to an uncontrolled activation of the complement system resulting in a depletion of serum complement. Factor H deficient patients are susceptible to recurrent bacterial infection (particularly meningitis) and may not be able to clear immune complexes efficiently from circulation, resulting in glomerulonephritis.

Factor H is an important complement regulator which controls activation by its virtue to bind to native and complexed C3b and to serve as a cofactor in the Factor I mediated conversion of C3b to haemolytically inactive iC3b (Whaley, K. and Ruddy, S., 1976, J. Exp. Med., 144: 1147). It hereby acts as an antagonist to factor B and holds in check the alternative pathway activation, a positive feedback loop in which C3b

complexes with factor B, after which the serine protease factor D activates factor B by proteolysis, to form the alternative pathway C3 convertase, C3bBb. Factor H has a further important regulatory function as it can accelerate the decay of the C3 convertase by displacing Bb from the complex (Whaley, K. and Ruddy, S., 1976, Science, 193: 1011). Absence of factor H results in uncontrolled turnover of the alternative pathway. Because C3b is an integral component of the C5 convertases of both classical and alternative pathways, the binding of factor H to C3b also regulates C5 convertase activity (Whaley, K. and Ruddy, S., 1976, Science, 193: 1011). Thus factor H plays a key role in controlling the alternative pathway C3 convertase activity and also the activities of the C5 convertases of both classical and alternative pathways.

No complement regulatory activity has as yet been ascribed to the recently characterized variant factor H related serum glycoproteins of 39/43 kDa and 24/29 kDa (Timmann, C. *et al.*, 1991, J. Immunol., 146:1265; Estaller, C. *et al.*, 1991, J. Immunol., 146: 3190; Schwaeble, W. *et al.*, 1991, Eur J. Biochem., 198: 399 - 404; Skerka, C. *et al.*, 1991, J. Biol. Chem., 266: 12015; Zipfel, P.F. and Skerka, C., 1994, Immunology Today, 15: 121). These factor H related mRNAs are exclusively expressed in the liver (Schwaeble, W. *et al.*, 1991, Immunobiol., 182:307) and encoded by at least two different factor H related genes (Estaller, C. *et al.*, 1991, J. Immunol., 146: 3190; Hourcade, D. *et al.*, 1991, Abstr. XIVth Int. Complement Workshop, Complement Inflamm., 8: 163; Zipfel, P.F. and Skerka, C., 1994, Immunology Today, 15: 121).

Factor H comprises a number of independently folded domains (CCP modules or short consensus repeats - SCRs) of approximately 60 amino acids (aa) residues with a framework of highly conserved residues involving 4 cysteine, 1 tryptophane and 2 proline residues. In human serum, two different FH glycoproteins of 155 kDa (FHp155) and of 43 kDa (FHp43) are known (Schwaeble, W. *et al.*, 1987, Eur. J. Immunol., 17: 1485; Ripoche, J. *et al.*, 2988, Biochem. J., 249: 593; Schwaeble, W. *et al.*, 1991, Eur. J. Biochem., 198: 399-404; Estaller, C. *et al.*, Eur. J. Immunol., 21:

799) and both forms express cofactor (i.e. complement regulatory) activity in the FI (Factor I) mediated conversion of C3b to iC3b (Misasi, R. *et al.*, 1989, Eur. J. Immunol., 19: 1765 - 1768). See also Whaley, K. and Ruddy, S., 1976, J. Exp. Med. 144: 1147-1163; Whaley, K. and Ruddy, S., 1976, Science, 193: 1011-1013.

According to the present invention there is provided a molecule comprising at least complement control protein (CCP) modules (Reid, K.B.M. *et al.*, 1986, Immunol. Today, 7: 230-234) 1-4 of complement factor H, or a molecule resulting from partial modification thereof or an allelic mutant thereof.

By "partial modification" and "partially modified" is meant, with reference to amino acid sequences a partially modified form of the molecule which retains substantially the properties of the molecule from which it is derived, although it may of course have additional functionality. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues. Substitutions may be conserved substitutions. Hence the partially modified molecules may be homologues of the molecules from which they are derived. They may, for example, have at least 40% homology with the molecules from which they are derived. They may for example have at least 50, 60, 70, 80, 90 or 95% homology with the molecules from which they are derived. Similarly nucleotide sequences encoding the molecules or amino acid sequences may be partially modified to code for any such modifications to an amino acid sequence or molecule. Nucleotide sequences may also of course be modified such that they still code for the same amino acid residues but have a different nucleotide sequence.

The molecule may for example comprise CCP modules 1-5, 1-6 or 1-7 of complement factor H, or a molecule resulting from partial modification thereof or an allelic mutant thereof.

The complement factor H may be human complement factor H or it may for example be a different animal complement factor H, for example rat complement factor H.

The molecule may comprise FHp43, or a molecule resulting from partial modification thereof or an allelic mutant thereof.

The molecule may be for use in inhibiting complement activation.

The present inventor has found that, surprisingly, FHp43 is approximately 10-100 fold more potent than FHp155, and that this potency is to be found particularly in CCP modules 1-7.

Hence a molecule according to the present invention may have increased complement inhibitory activity compared to that of FHp155, i.e. it may have an enhanced efficacy. A molecule according to the present invention comprises at least CCP modules 1-4 of FHp43. It may for example comprise at least CCP modules 1-5, 1-6 or 1-7 of FHp43.

The present inventor has found that the C-terminal 180 amino acids of FHp43 may be removed without significant loss of the complement inhibitory function of FHp43. Hence molecules according to the present invention may have C-terminal deletions of for example about 180 amino acids, when compared to FHp43.

The regulatory activity of these molecules may be used for example in preventing tissue damage due to myocardial infarction, ischemia (for example limb and gut ischemia), infarction of neural tissue, in treating the adult respiratory distress syndrome, rheumatoid arthritis and thermal injuries. The molecules may be used as a fluid phase regulator of complement activity. They may for example be used to improve

the biocompatability of artificial membranes by e.g. coating haemofiltration membranes with immobilised FH polypeptides in order to reduce complement activation or by encapsulating xenografts in artificial membranes coated with FH polypeptides. Fusion proteins may be made comprising a FH protein according to the present invention fused to a membrane anchor in order to act as a potent complement regulator on the surface of transfected (or transformed) cells and transgenic animals. Such membrane anchored molecules may be used to reduce xenograft rejection using xenotransplant organs. Spacer residues may be added between the membrane anchor and the FH protein in order to increase or optimise the efficacy of the FH protein (Adams, E.M. *et al.*, 1991, J. Immunol., 147: 3005). Methods of transformation and transfection of cells are well known in the art and where reference is made to transfection, reference is also to transformation and *vice versa*.

Molecules according to the present invention may be modified such that they have an increased half-life in order that they may have a prolonged protective effect upon a patient. Particular molecules may for example comprise dimeric or trimeric forms of molecules according to the present invention. For example a molecule may comprise a trimer of CCP modules 1-4 or a trimer of FHp43.

Also provided according to the present invention is the use of a molecule according to the present invention in the manufacture of a medicament for use in inhibiting complement activation.

Also provided according to the present invention is a method of inhibiting complement activation comprising the use of a molecule according to the present invention.

The present inventor has also succeeded in isolating and sequencing rat FH 4.3 and FH1.0 mRNA and so according to the present invention there is also provided

a nucleotide sequence having the sequence of SEQ ID NO: 1 (Figure 1 - FH4.3) encoding rat FH 4.3 kb mRNA, together with a nucleotide sequence having the sequence of SEQ ID NO: 2 (Figure 1 - FH1.0) encoding rat FH 1.0 kb mRNA. The present invention also extends to partially modified forms of the nucleotide sequences and to polypeptides derived from them and partially modified forms thereof.

FHp155 and FHp43 may be readily isolated and purified (Misasi, R. *et al.*, Eur. J. Immunol., 1989, 19: 1765-1768; Sim, R.B. *et al.*, 1993, Int. Rev. Immunol., 10: 65; Sim, R.B. *et al.*, 1993, Meth. Enzymol., 223: 13 and references therein) and the genes encoding the proteins may be isolated using standard techniques. Standard expression systems, for example MaxBac (Invitrogen) may be used to synthesise the isolated protein (see Sharma, A.K. and Pangburn, M.K., 1994, Gene, 143: 301).

The ability of the molecules of the present invention to inhibit complement activation may be readily shown by activating complement with antigen-antibody complexes (classical pathway) or zymosan (alternative pathway) in the presence of the molecules of the present invention and assaying levels of C3a, C5a and C5b-9 complement components using commercially available reagents (Amersham) and ELISA (enzyme linked immunosorbent assay).

The alternative pathway C3 and C5 convertases ($(C3b)_nBbP$) and classical pathway C5 convertase (C4b2a3b) may be readily prepared from for example rat or human components and the activity of the factor H molecules of the present invention on the formation and stability of each convertase and on C5 activation may be assayed using haemolytic assay systems (Sim *et al.*, 1993, *supra*).

The ability of the molecules of the present invention to inhibit complement activation and limit tissue injury *in vivo* may be determined using for example a model of perfusion injury of ischaemic myocardium (Weisman, H.F *et al.*, 1990, Science, 249:

146) and a model of antibody-dependent experimental allergic encephalomyelitis (Piddlesden, S. *et al.*, 1990, Clin. Exp. Immunol., 83: 245).

The molecules of the present invention may be readily coupled to artificial membranes, for example dialysis membranes, as follows. Using cuprophan-cellulose membranes (Enka-Azko, Wuppertal, Germany), the following steps may be performed:

i) Activation of the membrane:

1,1'-Carbodiimidazole (Kennedy, J.F. and Paterson, M., 1993, Polymer. Intern., 32: 71;

Chlorformic acid-p-nitrophenylester (Vandorne, F. *et al.*, 1991, Makromol. Chem., 192: 773);

Cyanogen bromide (Kennedy, J.F. and Patterson, M., 1993, *supra*)

ii) Coupling of spacers:

Use of aliphatic diamines (e.g. 1,12 Diaminododecane, Kery *et al.*, 1991, Carbohydr. Res., 209: 83);

Use of 6-aminocaproicacid (Burton, S.C., 1991, J. Chromatogr., 587: 271);

Use of aminosubstituted aliphatic thiols (Kery *et al.*, 1991, *supra*)

iii) Coupling of the peptide:

Activation of the N-terminal spacer by thiophosgen;

Activation of a carboxyterminal spacer using alternatively the acid method or the addition of coupling reagents (e.g. DCC or EDC, Royer, G.P. and Anantharmaiah, G.M., 1979, J. Am. Chem. Soc., 101: 3395; Bodanszky, M. and Bodanszky, A., 1984, K. Hafner *et al.*, Hrsg, Bd. 21, Springer-Verlach, Berlin);

Activation of S-terminal spacer by 2,2'-Dithiodipyridine and coupling via cysteine residues.

The effect of uncoated and coated membranes (above) upon complement activation may be readily quantified using C3a, C5a and C5b-9 assays (Chenoweth, D.E., 1987, Contr. Nephrol., 59: 51 and as described above).

According to a further aspect of the invention, there is provided a DNA molecule, which may be in recombinant or isolated form, comprising a sequence encoding a molecule according to the present invention.

The coding sequence may be operatively linked to an expression control sequence sufficient to drive expression. Recombinant DNA in accordance with the invention may be in the form of a vector. The vector may for example be a plasmid, cosmid or phage. A vector may include at least one selectable marker to enable selection of cells transfected (or transformed) with the vector. Such a marker or markers may enable selection of cells harbouring vectors incorporating heterologous DNA. The vector may contain appropriate start and stop signals. The vector may be an expression vector having regulatory sequences to drive expression. Vectors not having regulatory sequences may be used as cloning vectors (as may expression vectors).

Cloning vectors can be introduced into suitable hosts (for example *E. coli*) which facilitate their manipulation. According to another aspect of the invention, there is therefore provided a host cell transfected or transformed with DNA according to the present invention. Such host cells may be prokaryotic or eukaryotic. Eukaryotic hosts may include yeasts, insect and mammalian cell lines. Expression hosts may be stably transformed. Unstable and cell-free expression systems may of course also be used.

DNA of the invention may also be in the form of a transgene construct designed for expression in a transgenic plant or animal. In principle, the invention is applicable to all animals, including birds such as placental mammals, (for example cattle, sheep, goats, water buffalo, camels and pigs), domestic fowl, amphibian species and fish

species. The protein may be harvested from body fluids or other body products (such as eggs or milk, where appropriate). Such mammalian transgenic mammary expression systems are well known - see for example WO-A-8800239, WO-A-9005188 and WO-A-9416570. The β-lactoglobulin promoter may be used in transgenic mammary expression systems.

Expression hosts, particularly transgenic animals, may contain other exogenous DNA to facilitate the expression, assembly, secretion and other aspects of the biosynthesis of molecules of the invention.

The invention is in principle capable of accommodating the use of synthetic DNA sequences, cDNAs, full genomic sequences and "minigenes", i.e. partial genomic sequences containing some, but not all, of the introns present in the full length gene.

DNA in accordance with the invention can in principle be prepared by any convenient method involving coupling together successive nucleotides, and/or ligating oligo- and/or poly-nucleotides, including *in vitro* processes, as well as by the more usual recombinant DNA technology.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, forms of complement inhibition. Of the figures:

Figure 1 shows sequence alignments of the nucleotide sequences of four different types of rat factor H mRNA transcripts (rFH4.3, rFH2.7, rFH1.8 and rFH1.0). Start and stop-codons are underlined, the polyadenylation initiation signal is written in italics;

Figure 2 shows a cofactor assay showing the functional activity of recombinant human FHp43. Lanes are as follows: Lane 1 - C3b with human Factor I (FI); lane 2 - C3b with rat FI; lane 3 - C3b with human FI and recombinant rat FHSCR1-7; lane 4 - C3b with human FI and recombinant human FHp43 (10 mM); and lane 5 - C3b with rat FI and purified human factor H; and

Figure 3 shows a cofactor assay showing the functional activity of recombinant rat FHSCR1-7. Lanes are as follows: Lane 1 - C3b with human FI; lane 2 - C3b with rat FI; lane 3 - C3b with human FI and recombinant human factor H; lane 4 - C3b with human FI and recombinant rat factor H; lane 5 - C3b with rat FI and recombinant rat FHSCR1-7; lane 6 - C3b with rat factor I and 10 mM recombinant rat FHSCR1-7; and lane 7 - C3b with human factor I and 10 mM recombinant FHp43.

EXPERIMENTAL

With the following experiments, a truncated recombinant human and rat factor H are expressed in a high efficiency yeast expression system. The yield of expression is estimated to be in a range of up to 5mg of recombinant protein per litre of yeast culture.

Figures 2 and 3 show the results of the cofactor assays described below. The presence of an α' band at 43 kDa (a cleavage product of the α-chain of C3b) indicates cofactor activity (Figure 2, lane 4; Figure 3, lanes 3, 5, 6 and 7). Hence both the recombinant human FHp43 and rat FHSCR1-7 peptides cooperate with factor I in a species specific manner and, surprisingly, exhibit cofactor activity even at low concentrations (10 mM) when incubated with C3b and factor I of the corresponding species.

Materials and Methods

Isolation and characterization of 4 different factor H or factor H related gene products of the rat

Using a rat liver cDNA library in λ-ZAP II (#937506 STATAGENE, La Jolla, CA), cDNA clones rFH4.3, rFH1.8, rFH2.7 and rFH1.0 were isolated as follows. Approximately 300,000 colonies were screened with a 5' specific PstI/XhoI cDNA subfragment of the mouse factor H cDNA clone MH8 (Kirstensen, T. *et al.*, 1986, J. Immunol., 136: 3407). From eighteen hybridizing plaques obtained in the rescreen procedure, the four clones listed above were analysed further. The pBluescript SK- plasmid containing the cDNA insertions of interest were rescued from the λ-ZAP II phagemid by *in vivo* excision. The cDNA sequences of the 4 different types of clones was determined by sequencing both strands using the Sanger dideoxy chain termination method with Sequenase II (RTM) and the reagent kit (USB, Cleveland, USA).

RNA extraction and Northern blot analysis

Total RNA was isolated according to standard methods (Chirgwin, J.W. *et al.*, 1979, Biochemistry, 18: 5294), quantified by measuring the absorbence at 260 nm, separated on a formaldehyde-containing 1.2% agarose gel and blotted to Hybond N filters. Agarose gel electrophoresis, RNA transfer and hybridization of blots were performed by standard techniques (Sambrook, J., Frisch, E.F., and Maniatis, T.: Molecular cloning. A laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York, 1989). Northern blot filters were probed with a 5'-specific 553 bp long PstI/XhoI restriction subfragment of the murine factor H clone MH8 encoding SCR 1-2 of mouse factor H, and the 867 bp long cDNA insert of the rat specific factor H clone rFH1.0. The probes were used at a concentration of $5x10^6$ cpm of ^{32}P labelled cDNA/ml hybridization solution. Hybridization was performed at 65 °C in the absence of formamide. The washing of the Northern blots was carried out according to standard procedures (Sambrook et al., 1989, *supra*). The last washing step was performed in 0.3x SSC for 1 hour at 65 °C.

Expression of recombinant human and rat factor H in Pichia pastoris

The coding sequence for the mature human factor H serum protein FHp43 was amplified by PCR using the oligonucleotide primers H19 5' EcoRI: 3' GTA GAA TTC **GAA** GAT TGC AAT GAA CTT 5' and the reverse 3' primer H19 3' NOT I : 5' GGG CGG CCG **CTC AGA** GGG TAA AGC TGA C 3' using cDNA clone phFH1.8 (Estaller, C. *et al.*, 1991, Eur. J. Immunol., 21: 799) as template. Characters in bold indicate the start of the Factor H sequence or the end of the coding Factor H sequence as appropriate. Uppercase characters are coding and lowercase characters are non-coding. In order to obtain further truncated versions of recombinant factor H proteins (i.e. SCR1-6, SCR1-5, SCR1-4), the same procedure was repeated using the primers H19 5' EcoRI: 3' GTA GAA TTC **GAA** GAT TGC AAT GAA CTT 5' and the reverse 3' primer H19 3' SCR6 NOT I : 5' GGG CGG CCG **CTC A** tac tgg aaa gta tgg tct acg 3' (to amplify

SCR1-6), H19 5' EcoRI: 3' GTA GAA TTC **GAA** GAT TGC AAT GAA CTT 5' and the reverse 3' primer H19 3' SCR5 NOT I : 5' GGG CGG CCG **CTC A** ttt aat cct taa agg tga gta 3' (to amplify SCR1-5), H19 5' EcoRI: 3' GTA GAA TTC **GAA** GAT TGC AAT GAA CTT 5' and the reverse 3' primer H19 3' SCR4 NOT I : 5' GGG CGG CCG **CTC A** aat ctt ctg aga tat agg aga 3' (to amplify SCR1-4). In each case, the PCR reaction was performed using the GeneAmp DNA amplification reagent kit (Perkin Elmer Cetus, Überlingen , Germany) and the PCR protocol: 95 °C for 5 minutes, followed by 40 cycles (95 °C for 1 min., 50 °C for 2 min, 72 °C for 2 min.) and 72 °C for 10 min. The PCR products were subcloned into the PCRII (INVITROGEN, San Diego, CA), excised using the EcoRI/NotI restriction sites generated within the primers and cloned in frame with the alpha factor prepro sequence of the Pichia pastoris expression vector pPICZα A (INVITROGEN, San Diego, CA) (using the EcoRI/NotI restriction sites in the polylinker of pPICZα A).

Likewise, the first seven SCR units of rat factor H were amplified by PCR using the oligonucleotide primers rFH4.3-5' *Sna* B I: 3' GGT ACG TAG AAG ATT GTA AAG GTC CT 5' and rFH4.3-3' *Not* I 3' GGG CGG CCG CGA TAC GGA CGC ATT TGG G 5' with cDNA clone rFH4.3 as a template. The PCR product was subcloned into PCRII, excised using the SnaBI and the NotI restriction sites introduced within the primers and subcloned in frame with the alpha factor prepro coding sequence using the corresponding restriction sites of the Pichia pastoris expression vector pPIC 9 (INVITROGEN, San Diego, CA). The ligation products were transfected and amplified in the *E. coli* strain TOP 10 (INVITROGEN, San Diego, CA) according to the manufacturers protocol.

The Pichia pastoris strain GS115 was transfected with the linearised constructs (the pPICZα construct containing the human factor H cDNA was linearised by BstX1 digest, the pPIC9 construct containing the rat factor H cDNA was linearised by Bgl II digest)

electroporation using the BioRad Gene Pulsar (BioRad, Hercules, CA) according to the manufacturers protocol. Plating and screening for transformants was performed according to the manufacturers protocol (INVITROGEN, San Diego, CA). After electroporation, Pichia pastoris cells were plated on MD plates (containing dextrose) and grown at 30 °C for 48 hours. Single colonies were picked from these plates and replated on Methanol containing MM plates (without dextrose) to select for AOX1- disrupted transformants which have the cDNA of interest inserted into the polylinker region. Alcohol oxidase genes AOX1 and AOX2 allow the metabolism of methanol, thereby providing a source of carbohydrates. MM plates (without dextrose) provide no other source of carbohydrates and so AOX1-disrupted transformants, which have a reduced ability to metabolise methanol, were recognised by their slower growth on dextrosol-free MM plates. The insertion of the cDNA construct of interest was further confirmed by PCR analysis of genomic DNA isolated from poorly growing colonies. In order to select for such colonies that secrete high rates of recombinant factor H, twenty AOX1-disrupted colonies were inoculated each in 10 ml of BMGY medium (Invitrogen) in a 50 ml tube and cultured at 30 °C with vigorous shaking (>200 rpm) for 48 hours to saturation (OD_{600} = 10.0-20.0). Cells were harvested by centrifugation for 10 minutes at room temperature at 4000 g, supernatant discarded and the pellet resuspended in 2 ml of BMMY (Invitrogen) medium. This time, tubes were only covered with two layers of sterile gauze and again, incubation occurred at 30 °C with vigorous shaking (>200 rpm) for 48 hours. Cells were pelleted as before and supernatants analysed by Western blot analysis.

Cofactor assay

Functional activity of recombinant rat and human factor H was determined in a factor H dependent factor I mediated C3b cleavage assay. Therefore, human C3b and factor I were purified from peripheral blood as previously described (Misasi, R. *et al.*, 1989, Eur. J. Immunol., 19: 1765). In order to establish a species-specific variant of this assay, rat factor I was purified from 2 ml of rat serum by fluid phase liquid chromatography using Pharmacia FPLC apparatus P500 and a Pharmacia Mono S HR 5/5 column eqilibrated

with PE buffer at pH 6. Separation of serum proteins occurred by addition of PE-buffer plus 1M NaCl at pH 6 and a flow rate of 1 ml/min. Fractions were depleted of factor H by immune-chromatography using a Sepharose C14b column preabsorbed with the human anti-factor H monoclonal antibody OX23 (Schwaeble, W. *et al.*, 1987, Eur. J. Immunol., 17: 1485). The cofactor assay for the recombinant human FHp43 and rat FHSCR1-7 expressed in yeast as described above was performed in a 1.5 ml Eppendorf reaction tube at 37 °C for 30 min using 100,000 cpm of ^{125}I labelled C3b diluted in PE buffer with 20 mg SBTI, 0.1% Triton X 100, pH 7 by addition of either 1 µg rat or human factor I alone or 1 µg of recombinant rat FHSCR1-7 or human FHp43 alone or combinations of human factor I with rat or human recombinant factor H or rat factor I with recombinant human or rat factor H. Cleavage of C3b was monitored by SDS-PAGE and autoradiography by the generation of the 73 kDa and 43 kDa cleavage products of the α-chain of C3b. Production of the 43 kDa α' cleavage product was indicative of cofactor activity.

CLAIMS

1. A molecule comprising at least complement control protein modules 1-4 of complement factor H, or a molecule resulting from partial modification thereof, or an allelic mutant thereof.

2. A molecule according to claim 1 comprising complement control protein modules 1-5, 1-6 or 1-7 of complement factor H, or a molecule resulting from partial modification thereof, or an allelic mutant thereof.

3. A molecule according to either one of claims 1 or 2, the complement factor H being human complement factor H.

4. A molecule according to any one of claims 1-3, comprising Fhp43 or a molecule resulting from partial modification thereof, or an allelic mutant thereof.

5. A molecule according to any one of claims 1-4, for use in inhibiting complement activation.

6. A molecule according to claim 5, having an enhanced efficacy when compared to FHp155.

7. The use of a molecule according to any one of the preceding claims in the manufacture of a medicament for inhibiting complement activation.

8. A method of inhibiting complement activation comprising the use of a molecule according to any one of claims 1-6.

9. A nucleotide sequence having the formula of SEQ ID NO: 1 and encoding rat FH 4.3 kb mRNA.

10. A nucleotide sequence having the formula of SEQ ID NO: 2 and encoding rat FH 1.0 mRNA.

11. A DNA molecule comprising a sequence encoding a molecule according to any one of claims 1-6.

ABSTRACT

The present invention concerns regulation of complement activation, in particular the fluid phase regulation of complement activation, and provides molecules comprising at least complement control protein modules 1-4 of complement factor H, DNA molecules encoding same, their use in the manufacture of a medicament for inhibiting complement activation and methods of same, together with DNA sequences encoding rat FH 4.3 and 1.0 kb mRNA.

Figure 1

```
          10         20         30 -18     40         50         60
tcgagtcaactgctcccagatagatccaagacATGAGACTGTCAGCAAGAATTATTTGGC    rFH4.3
tcgagtcaactgctcccagatagatccaagacATGAGACTGTCAGCAAGAATTATTTGGC    rFH2.7
tcgagtcaactgctcccagatagatccaagacATGAGACTGTCAGCAAGAATTATTTGGC    rFH1.8
tcgagtcaactgctcccagatagatccaagacATGAGACTGTCAGCAAGAATTATTTGGC    rFH1.0

                              SCR1
          70         80        +1 90         100        110        120
TTATATTATGGACTGTTTGTGTAGCAGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAATT    rFH4.3
TTATATTATGGACTGTTTGTGTAGCAGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAATT    rFH2.7
TTATATTATGGACTGTTTGTGTAGCAGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAATT    rFH1.8
TTATATTATGGACTGTTTGTGTAGCAGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAATT    rFH1.0

          130        140        150        160        170        180
CAGAAATTCTCTCAGGTTCGTGGTCTGAACAACTATATTCAGAAGGCACTCAGGCAACCT    rFH4.3
CAGAAATTCTCTCAGGTTCGTGGTCTGAACAACTATATTCAGAAGGCACTCAGGCAACCT    rFH2.7
CAGAAATTCTCTCAGGTTCGTGGTCTGAACAACTATATTCAGAAGGCACTCAGGCAACCT    rFH1.8
CAGAAATTCTCTCAGGTTCGTGGTCTGAACAACTATATTCAGAAGGCACTCAGGCAACCT    rFH1.0

          190        200        210        220        230        240
ACAAATGCCGCCCTGGATACCGAACACTTGGTACTATTGTAAAAGTATGCAAGAATGGAG    rFH4.3
ACAAATGCCGCCCTGGATACCGAACACTTGGTACTATTGTAAAAGTATGCAAGAATGGAG    rFH2.7
ACAAATGCCGCCCTGGATACCGAACACTTGGTACTATTGTAAAAGTATGCAAGAATGGAG    rFH1.8
ACAAATGCCGCCCTGGATACCGAACACTTGGTACTATTGTAAAAGTATGCAAGAATGGAG    rFH1.0

                                   SCR2a
          250        260        270        280        290        300
AATGGGTACCTTCTAACCCATCAAGGATATGTCGGAAAAGGCCATGTGGGCATCCCGGAG    rFH4.3
AATGGGTACCTTCTAACCCATCAAGGATATGTCGGAAAAGGCCATGTGGGCATCCCGGAG    rFH2.7
AATGGGTACCTTCTAACCCATCAAGGATATGTCGGAAAAGGCCATGTGGGCATCCCGGAG    rFH1.8
AATGGGTACCTTCTAACCCATCAAGGATATGTCGGAAAAGGCCATGTGGGCATCCCGGAG    rFH1.0

          310        320        330        340        350        360
ACACACCCTTTGGGTCCTTTAGGCTGGCAGTTGGATCTGAATTTGAATTTGGTGCAAAGG    rFH4.3
ACACACCCTTTGGGTCCTTTAGGCTGGCAGTTGGATCTGAATTTGAATTTGGTGCAAAGG    rFH2.7
ACACACCCTTTGGGTCCTTTAGGCTGGCAGTTGGATCTGAATTTGAATTTGGTGCAAAGG    rFH1.8
ACACACCCTTTGGGTCCTTTAGGCTGGCAGTTGGATCTGAATTTGAATTTGGTGCAAAGG    rFH1.0
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                    SCR2b
         370        380        390        400        410        420
TTGTTTATACATGTGATGAAGGGTACCAACTATTAGGTGAAATTGATTACCGTGAATGTG    rFH4.3
TTGTTTATACATGTGATGAAGGGTACCAACTATTAGGTGAAATTGATTACCGTGAATGTG    rFH2.7
TTGTTTATACATGTGATGAAGGGTACCAACTATTAGGTGAAATTGATTACCGT-------    rFH1.8
TTGTTTATACATGTGATGAAGGGTACCAACTATTAGGTGAAATTGATTACCGTGAATGTG    rFH1.0

                                  SCR3
         430        440        450        460        470        480
ATGCAGATGGGTGGACCAATGATATTCCAATATGTGAAGTTGTGAAGTGCTTGCCAGTGA    rFH4.3
ATGCAGATGGGTGGACCAATGATATTCCAATATGTGAAGTTGTGAAGTGCTTGCCAGTGA    rFH2.7
------------------------------------------------------------    rFH1.8
ATGCAGATGGGTGGACCAATGATATTCCAATATGTGAAGTTGTGAAGTGCTTGCCAGTGA    rFH1.0

         490        500        510        520        530        540
CAGAACTGGAGAATGGAAGAATTGTGAGTGGTGCAGCCGAACCAGACCAGGAATATTATT    rFH4.3
CAGAACTGGAGAATGGAAGAATTGTGAGTGGTGCAGCCGAACCAGACCAGGAATATTATT    rFH2.7
------------------------------------------------------------    rFH1.8
CAGAACTGGAGAATGGAAGAATTGTGAGTGGTGCAGCCGAACCAGACCAGGAATATTATT    rFH1.0

         550        560        570        580        590        600
TTGGACAGGTGGTACGCTTTGAATGCAACTCCGGCTTCAAGATTGAAGGACAGAAAGAAA    rFH4.3
TTGGACAGGTGGTACGCTTTGAATGCAACTCCGGCTTCAAGATTGAAGGACAGAAAGAAA    rFH2.7
------------------------------------------------------------    rFH1.8
TTGGACAGGTGGTACGCTTTGAATGCAACTCCGGCTTCAAGATTGAAGGACAGAAAGAAA    rFH1.0

                                            SCR4
         610        620        630        640        650        660
TGCACTGCTCATAAAATGGCCTCTGGAGCAATGAAAAGCCACAGTGTGTGGAAATTTCTT    rFH4.3
TGCACTGCTCATAAAATGGCCTCTGGAGCAATGAAAAGCCACAGTGTGTG----------    rFH2.7
------------------------------------------------------------    rFH1.8
TGCACTGCTCATAAAATGGCCTCTGGAGCAATGAAAAGCCACAGTGTGTGGAAATTTCTT    rFH1.0

         670        680        690        700        710        720
GCCTGCCACCACGAGTTGAAAATGGAGATGGTATATATCTGAAACCAGTTTACAAGGAGA    rFH4.3
------------------------------------------------------------    rFH2.7
------------------------------------------------------------    rFH1.8
GCCTGCCACCACGAGTTGAAAATGGAGAT------------------------------    rFH1.0
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        730        740        750        760        770        780
ATGAAAGATTCCAATATAAATGTAAGCAAGGTTTTGTGTACAAAGAAAGAGGGGATGCTG   rFH4.3
------------------------------------------------------------   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                    SCR5
        790        800        810        820        830        840
TCTGCACGGGTTCTGGATGGAATCCTCAGCCTTCCTGTGAAGAAATGACATGTTTGACTC   rFH4.3
------------------------------------------------------------   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        850        860        870        880        890        900
CATATATTCCAAATGGTATCTACACACCTCACAGGATTAAACACAGAATTGATGATGAAA   rFH4.3
------------------------------------------------------------   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        910        920        930        940        950        960
TCAGATATGAATGTAAAAATGGCTTCTATCCTGCAACCCGATCACCTGTTTCAAAGTGTA   rFH4.3
------------------------------------------------------------   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                    SCR6
        970        980        990       1000       1010       1020
CAATTACTGGCTGGATCCCTGCTCCAAGATGTAGCTTGAAACCTTGTGATTTTCCACAAT   rFH4.3
----------------------------------TTGAAACCTTGTGATTTTCCACAAT   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

       1030       1040       1050       1060       1070       1080
TCAAACATGGACGTCTGTATTATGAAGAAAGCCGGAGACCCTACTTCCCAGTACCTATAG   rFH4.3
TCAAACATGGACGTCTGTATTATGAAGAAAGCCGGAGACCCTACTTCCCAGTACCTATAG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0
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        1090       1100       1110       1120       1130       1140
GAAAGGAGTACAGCTATAACTGTGACAACGGGTTTACAACGCCTTCACAGTCATACTGGG   rFH4.3
GAAAGGAGTACAGCTATAACTGTGACAACGGGTTTACAACGCCTTCACAGTCATACTGGG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                      SCR7
        1150       1160       1170       1180       1190       1200
ACTACCTTCGTTGCACAGTAAATGGGTGGGAGCCTGAAGTTCCATGCCTCAGGCAATGTA   rFH4.3
ACTACCTTCGTTGCACAGTAAATGGGTGGGAGCCTGAAGTTCCATGCCTCAGGCAATGTA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        1210       1220       1230       1240       1250       1260
TTTTCCATTATGTGGAATATGGAGAATCTTCATACTGGCAAAGAAGATATATAGAGGGTC   rFH4.3
TTTTCCATTATGTGGAATATGGAGAATCTTCATACTGGCAAAGAAGATATATAGAGGGTC   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        1270       1280       1290       1300       1310       1320
AGTCTGCAAAAGTCCAGTGTCACAGTGGCTATAGTCTTCCAAATGGTCAAGATACATATT   rFH4.3
AGTCTGCAAAAGTCCAGTGTCACAGTGGCTATAGTCTTCCAAATGGTCAAGATACATATT   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                      SCR8
        1330       1340       1350       1360       1370       1380
ATTGTACAGAGAATGGCTGGTCCCCTCCTCCCAAATGCGTCCGTATCAAGACTTGTTCAG   rFH4.3
ATTGTACAGAGAATGGCTGGTCCCCTCCTCCCAAATGCGTCCGTATCAAGACTTGTTCAG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        1390       1400       1410       1420       1430       1440
TATCAGATATAGAAATTGAAAATGGGTTTTTTTCTGAATCTGATTATACATATGCTCTAA   rFH4.3
TATCAGATATAGAAATTGAAAATGGGTTTTTTTCTGAATCTGATTATACATATGCTCTAA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0
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         1450       1460       1470       1480       1490       1500
ATAGAAAAACACGGTATAGATGTAAACAGGGATATGTAACAAATACCGGAGAAATATCAG    rFH4.3
ATAGAAAAACACGGTATAGATGTAAACAGGGATATGTAACAAATACCGGAGAAATATCAG    rFH2.7
------------------------------------------------------------    rFH1.8
                                                                rFH1.0

                                                        SCR9
         1510       1520       1530       1540       1550       1560
GAATAATTACTTGTCTTCAAGATGGATGGTCACCTCGACCCTCATGCATTAAGTCTTGTG    rFH4.3
GAATAATTACTTGTCTTCAAGATGGATGGTCACCTCGACCCTCATGCATTAAGTCTTGTG    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         1570       1580       1590       1600       1610       1620
ATATGCCTGTATTTGAGAATTCTATGACTAAGAATAATAACACATGGTTTAAACTCAATG    rFH4.3
ATATGCCTGTATTTGAGAATTCTATGACTAAGAATAATAACACATGGTTTAAACTCAATG    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         1630       1640       1650       1660       1670       1680
ACAAATTAGACTATGAATGTCACATTGGATATGAAAATGAATATAAACATACCAAAGGCT    rFH4.3
ACAAATTAGACTATGAATGTCACATTGGATATGAAAATGAATATAAACATACCAAAGGCT    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

                                                       SCR10
         1690       1700       1710       1720       1730       1740
CTATAACATGTACTTATGATGGATGGTCTAGTACACCCTCCTGTTATGAAAGAGAATGCA    rFH4.3
CTATAACATGTACTTATGATGGATGGTCTAGTACACCCTCCTGTTATGAAAGAGAATGCA    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         1750       1760       1770       1780       1790       1800
GCATTCCCCTGTTACACCAAGACTTAGTTGTTTTTCCCAGAGAAGTAAAATACAAAGTTG    rFH4.3
GCATTCCCCTGTTACACCAAGACTTAGTTGTTTTTCCCAGAGAAGTAAAATACAAAGTTG    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0
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        1810       1820       1830       1840       1850       1860
GAGATTCGTTGAGTTTCTCTTGCCGTTCAGGACACAGAGTTGGAGCAGATTTAGTGCAAT   rFH4.3
GAGATTCGTTGAGTTTCTCTTGCCGTTCAGGACACAGAGTTGGAGCAGATTTAGTGCAAT   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                    SCR11
        1870       1880       1890       1900       1910       1920
GCTACCACTTTGGATGGTCCCCTAATTTCCCAACGTGTGAAGGCCAAGTAAAATCATGTG   rFH4.3
GCTACCACTTTGGATGGTCCCCTAATTTCCCAACGTGTGAAGGCCAAGTAAAATCATGTG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        1930       1940       1950       1960       1970       1980
ACCAACCTCTTGAAATCCCGAATGGGGAAATAAAGGGAACAAAAAAAGTTGAATACAGCC   rFH4.3
ACCAACCTCTTGAAATCCCGAATGGGGAAATAAAGGGAACAAAAAAAGTTGAATACAGCC   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        1990       2000       2010       2020       2030       2040
ATGGTGACGTGGTGGAATATGATTGCAAACCTAGATTTCTACTGAAGGGACCCAATAAAA   rFH4.3
ATGGTGACGTGGTGGAATATGATTGCAAACCTAGATTTCTACTGAAGGGACCCAATAAAA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                    SCR12
        2050       2060       2070       2080       2090       2100
TCCAGTGTGTTGACGGGAAGTGGACAAGGTTGCCGATATGCGTTGAGTATGAGAGAACAT   rFH4.3
TCCAGTGTGTTGACGGGAAGTGGACAAGGTTGCCGATATGCGTTGAGTATGAGAGAACAT   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        2110       2120       2130       2140       2150       2160
GTGGAGACCTTCCTGAACTTGAGCATGGCTCTGTCAAGTTATCTGTCCCTCCCTACCATC   rFH4.3
GTGGAGACCTTCCTGAACTTGAGCATGGCTCTGTCAAGTTATCTGTCCCTCCCTACCATC   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0
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        2170       2180       2190       2200       2210       2220
ATGGAGATTCAGTGGAGTTCACTTGTACAGAAACCTTCACAATGATTGGACATGCAGTAG   rFH4.3
ATGGAGATTCAGTGGAGTTCACTTGTACAGAAACCTTCACAATGATTGGACATGCAGTAG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                    SCR13
        2230       2240       2250       2260       2270       2280
TTTTCTGCATTAGTGGAAGGTGGACCGAGCTTCCTCAATGTGTTGCAACAGATCAACTGG   rFH4.3
TTTTCTGCATTAGTGGAAGGTGGACCGAGCTTCCTCAATGTGTTGCAACAGATCAACTGG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        2290       2300       2310       2320       2330       2340
AGAAGTGTAAAGCCCCGAAGTCAACTGGCATAGATGCAATTCATCCAAATAAGAATGAAT   rFH4.3
AGAAGTGTAAAGCCCCGAAGTCAACTGGCATAGATGCAATTCATCCAAATAAGAATGAAT   rFH2.7
------------------------------------------------------------   rFH1.8
                                                               rFH1.0

        2350       2360       2370       2380       2390       2400
TTAATCATAACTTTAGTGTGAGTTACAGATGTAGACAAAAGCAGGAGTATGAACATTCAA   rFH4.3
TTAATCATAACTTTAGTGTGAGTTACAGATGTAGACAAAAGCAGGAGTATGAACATTCAA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

                                                    SCR14
        2410       2420       2430       2440       2450       2460
TCTGCATCAATGGAAGATGGGATCCTGAACCAAACTGTACAAGCAAAAGATTCTGCCCTC   rFH4.3
TCTGCATCAATGGAAGATGGGATCCTGAACCAAACTGTACAAGCAAAAGATTCTGCCCTC   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        2470       2480       2490       2500       2510       2520
CTCCCCCGCAGATTCCAAATGCCCAAGTGATTGAAACCACCGTGAAATACTTGGATGGAG   rFH4.3
CTCCCCCGCAGATTCCAAATGCCCAAGTGATTGAAACCACCGTGAAATACTTGGATGGAG   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0
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         2530       2540       2550       2560       2570       2580
AAAAAGTATCTGTTCTTTGCCAAGATGGTTACCTAACTCAGGGCCCAGAAGAAATGGTGT    rFH1.8
AAAAAGTATCTGTTCTTTGCCAAGATGGTTACCTAACTCAGGGCCCAGAAGAAATGGTGT    rFH2.7
------------------------------------------------------------    rFH4.3
------------------------------------------------------------    rFH1.0

                                        SCR15
         2590       2600       2610       2620       2630       2640
GTAAACATGGAAGGTGGCAGTCGTTACCACGCTGCACGGAAAAAATTCCATGTTCCCAGC    rFH4.3
GTAAACATGGAAGGTGGCAGTCGTTACCACGCTGCACGGAAAAAATTCCATGTTCCCAGC    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         2650       2660       2670       2680       2690       2700
CCCCTAAAATTGAACATGGATCTATTAAGTCGCCCAGGTCCTCAGAAGAGAGGAGAGATT    rFH4.3
CCCCTAAAATTGAACATGGATCTATTAAGTCGCCCAGGTCCTCAGAAGAGAGGAGAGATT    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         2710       2720       2730       2740       2750       2760
TAATTGAGTCCAGCAGTTATGAACACGGAACTACATTCAGCTATTGCTGTAGAGATGGAT    rFH4.3
TAATTGAGTCCAGCAGTTATGAACACGGAACTACATTCAGCTATTGCTGTAGAGATGGAT    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

         2770       2780       2790       2800       2810       2820
TCAAGATATCTGAAGAAAATAGGGTAACCTGCAACATGGGAAAATGGAGCTCTCTGCCTC    rFH4.3
TCAAGATATCTGAAGAAAATAGGGTAACCTGCAACATGGGAAAATGGAGCTCTCTGCCTC    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0

              SCR16
         2830       2840       2850       2860       2870       2880
GTTGTGTTGGAATACCTTGTGGACCCCCACCTTCAATTCCTCTTGGTATTGTTTCTCATG    rFH4.3
GTTGTGTTGGAATACCTTGTGGACCCCCACCTTCAATTCCTCTTGGTATTGTTTCTCATG    rFH2.7
------------------------------------------------------------    rFH1.8
------------------------------------------------------------    rFH1.0
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        2890       2900       2910       2920       2930       2940
AACTAGAAAGTTACCAATATGGAGAGGAGGTTACATACAATTGTTCTGAAGGCTTTGGAA   rFH4.3
AACTAGAAAGTTACCAATATGGAGAGGAGGTTACATACAATTGTTCTGAAGGCTTTGGAA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        2950       2960       2970       2980       2990       3000
TTGATGGACCAGCATTTATTAAATGTGTAGGAGGACAGTGGTCTGAACCTCCCAAATGCA   rFH4.3
TTGATGGACCAGCATTTATTAAATGTGTAGGAGGACAGTGGTCTGAACCTCCCAAATGCA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

   SCR17
        3010       3020       3030       3040       3050       3060
TAAAAACTGATTGTGACAACTTGCCCACATTTGAAATTGCCAAACCGACAGAAAAGAAAA   rFH4.3
TAAAAACTGATTGTGACAACTTGCCCACATTTGAAATTGCCAAACCGACAGAAAAGAAAA   rFH2.7
------------------------------------------------------------   rFH1.8
------------------------------------------------------------   rFH1.0

        3070       3080       3090       3100       3110       3120
AAAAATCATACAGGTCAGGAGAACAAGTGACATTCAGATGTCCACCTCCGTATCGAATGG   rFH4.3
------------------------------------------------------------   rFH2.7
----------------------------------------------TATCGAATGG   rFH1.8
------------------------------------------------------------   rFH1.0

        3130       3140       3150       3160       3170       3180
ATGGCTCTGACATTGTCACATGTGTTAATACGAAGTGGATTGGACAGCCGGTATGCAAAG   rFH4.3
------------------------------------------------------------   rFH2.7
ATGGCTCTGACATTGTCACATGTGTTAATACGAAGTGGATTGGACAGCCGGTATGCAAAG   rFH1.8
------------------------------------------------------------   rFH1.0

   SCR18
        3190       3200       3210       3220       3223       3240
ATAATTCCTGTGTGAATCCACCACATGTGCCAAATGCTACTATACTAACAAGGCACAAGA   rFH4.3
------------------------------------------------------------   rFH2.7
ATAATTCCTGTGTGAATCCACCACATGTGCCAAATGCTACTATACTAACAAGGCACAAGA   rFH1.8
------------------------------------------------------------   rFH1.0
```

```
         3250       3260       3270       3280       3290       3300
CTAAATATCCATCTGGTGACAAAGTACGTTATGACTGTAATAAACCTTTTGAATTATTTG    rFH4.3
-------------------------------------------------------------   rFH2.7
CTAAATATCCATCTGGTGACAAAGTACGTTATGACTGTAATAAACCTTTTGAATTATTTG    rFH1.8
-------------------------------------------------------------   rFH1.0

         3310       3320       3330       3340       3350       3360
GGGAAGTGGAAGTGATGTGCCAAAACGGGATTTGGACAGAACCACCGAAATGCAAAGATT    rFH4.3
-------------------------------------------------------------   rFH2.7
GGGAAGTGGAAGTGATGTGCCAAAACGGGATTTGGACAGAACCACCGAAATGCAAAGATT    rFH1.8
-------------------------------------------------------------   rFH1.0

  SCR19
         3370       3380       3390       3400       3410       3420
CAACAGGGAAATGTGGGCCTCCTCCACCTATTGACAATGGAGACATCACCTCCTTGTCAT    rFH4.3
-------------------------------------------------------------   rFH2.7
CAACAGGGAAATGTGGGCCTCCTCCACCTATTGACAATGGAGACATCACCTCCTTGTCAT    rFH1.8
-------------------------------------------------------------   rFH1.0

         3430       3440       3450       3460       3470       3480
TACCAGTATATGCACCATTATCATCAGTTGAATATCAATGCCAGAACTATTATCTACTTA    rFH4.3
-------------------------------------------------------------   rFH2.7
TACCAGTATATGCACCATTATCATCAGTTGAATATCAATGCCAGAACTATTATCTACTTA    rFH1.8
-------------------------------------------------------------   rFH1.0

         3490       3500       3510       3520       3530       3540
AGGGAAATAAGATAGTAACATGTAGAAATGGAAAGTGGTCTCAGCCACCAACCTGCTTAC    rFH4.3
-------------------------------------------------------------   rFH2.7
AGGGAAATAAGATAGTAACATGTAGAAATGGAAAGTGGTCTCAGCCACCAACCTGCTTAC    rFH1.8
-------------------------------------------------------------   rFH1.0

  SCR20
         3550       3560       3570       3580       3590       3600
ATGCATGTGTGATACCAGAAGATATTATGGAAAAACATAATATAGTTCTCAGATGGAGGG    rFH4.3
-------------------------------------------------------------   rFH2.7
ATGCATGTGTGATACCAGAAGATATTATGGAAAAACATAATATAGTTCTCAGATGGAGGG    rFH1.8
-------------------------------------------------------------   rFH1.0
```

```
         3610       3620       3630       3640       3650       3660
AAAATGCAAAGATTTATTCCCAATCAGGGGAGAATATTGAATTCATGTGTAAACCTGGAT    rFH4.3
------------------------------------------------------------    rFH2.7
AAAATGCAAAGATTTATTCCCAATCAGGGGAGAATATTGAATTCATGTGTAAACCTGGAT    rFH1.8
-----------------------------------------------------GGAT    rFH1.0

         3670       3680       3690       3700       3710       3720
ATAGAAAATTCAGAGGATCACCTCCGTTTCGTACAAAGTGCATTGAGGGTCACATCAATT    rFH4.3
------------------------------------------------------------    rFH2.7
ATAGAAAATTCAGAGGATCACCTCCGTTTCGTACAAAGTGCATTGAGGGTCACATCAATT    rFH1.8
ATAGAAAATTCAGAGGATCACCTCCGTTTCGTACAAAGTGCATTGAGGGTCACATCAATT    rFH1.0

         3730       3740       3750       3760       3770       3780
ATCCCACTTGTGTATAAaatcgctatacaattattagtaaaccttatggatgagaaatgc    rFH4.3
------------------------------------------------------------    rFH2.7
ATCCCACTTGTGTATAAaatcgctatacaattattagtaaaccttatggatgagaaatgc    rFH1.8
ATCCCACTTGTGTATAAaatcgctatacaattattagtaaaccttatggatgacactttg    rFH1.0

         3790       3800       3810       3820       3830       3840
acatgtatattactaatacagtttgaatttacatttaaatattgtttagctcatttcctc    rFH4.3
------------------------------------------------------------    rFH2.7
acatgtatattactaatacagtttgaatttacatttaaatattgtttagctcatttcctc    rFH1.8
tttagaaatgcacatgtatattactaatacagtttgaatttacatttgaaaaa-------    rFH1.0

         3850       3860       3870       3880       3890       3900
taataagtatataaacttttttatatggtggttaatcagtaactttacagactgttgcc    rFH4.3
------------------------------------------------------------    rFH2.7
taataagtatataaacttttttatatggtggttaatcagtaactttacagactgttgcc    rFH1.8
------------------------------------------------------------    rFH1.0
```

```
        3910       3920       3930       3940       3950       3960
acaaagcaagaacattacattcaaaactcctaatccaaatatgatatgtccaaggacaaa   rFH4.3
-------------------------------------------------------------   rFH2.7
acaaagcaagaacattacattcaaaactcctaatccaaatatgatatgtccaaggacaaa   rFH1.8
-------------------------------------------------------------   rFH1.0

        3970       3980       3990       4000       4010       4020
ctatgtctaagcaagaaaataaatgttagttcttcaatgtctgtttttattcaggacctt   rFH4.3
-------------------------------------------------------------   rFH2.7
ctatgtctaagcaagaaaataaatgttagttcttcaatgtctgtttttattcaggacctt   rFH1.8
-------------------------------------------------------------   rFH1.0

        4030       4040       4050       4060       4070       4080
tcagattttcttggataccttttgttaggttctgattcacagtgagtggaagacacactg   rFH4.3
-------------------------------------------------------------   rFH2.7
tcagattttcttggataccttttgttaggttctgattcacagtgagtggaagacacactg   rFH1.8
-------------------------------------------------------------   rFH1.0

        4090       4100       4110       4120       4130       4140
actctgacttcaaattagtattacttgcaatacattaacaaccaaactatcataatatca   rFH4.3
-------------------------------------------------------------   rFH2.7
actctgacttcaaattagtattacttgcaatacattaacaaccaaactatcataatatca   rFH1.8
-------------------------------------------------------------   rFH1.0

        4150       4160       4170       4180       4190       4200
caaatgtatacagctaattactgtgtcctacctttgtatcaataaagaaatctaagaaag   rFH4.3
-------------------------------------------------------------   rFH2.7
caaatgtatacagctaattactgtgtcctacctttgtatcaataaagaaatctaagaaag   rFH1.8
-------------------------------------------------------------   rFH1.0

        4210       4220       4230
ttcttgcttaaaaaaaaaaaaaaaaaaa                                    rFH4.3
---------------------------                                     rFH2.7
ttcttgcttaaaaaaaaaaaaaaaaaaa                                    rFH1.8
---------------------------                                     rFH1.0
```

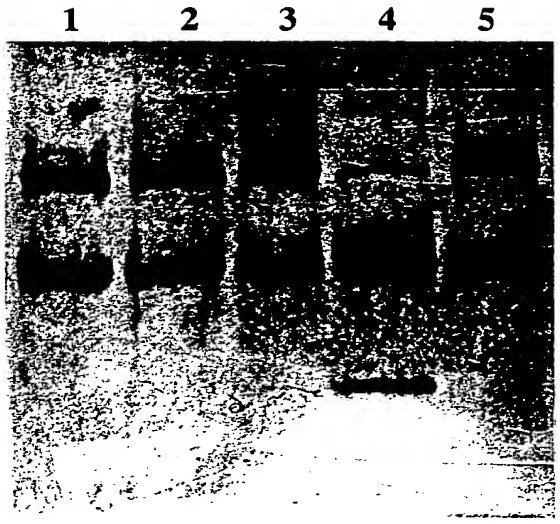

Figure 2

Spare

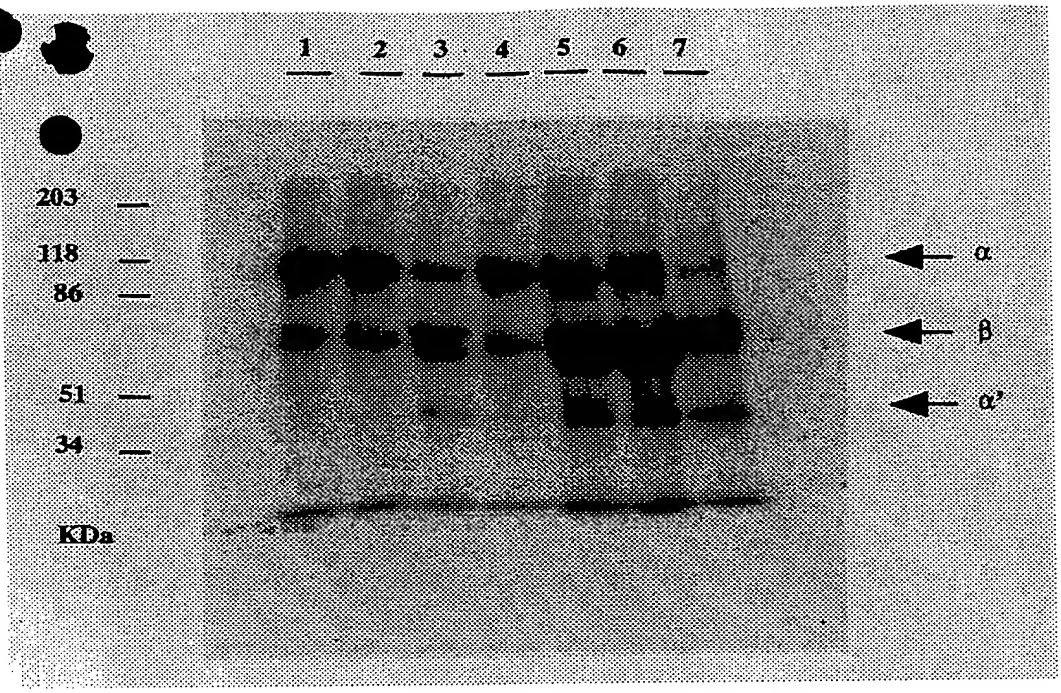

Figure 3

Spare